# Viral meningitis – current issues in diagnosis and treatment.

Fiona McGill1,2,3,4, Michael Griffiths1,2,5, Tom Solomon1,2,3,6

## Affiliations

Institute of Infection and Global Health, University of Liverpool, Liverpool, UK; 2.National Institute for Health Research Health Protection Research Unit on Emerging and Zoonotic Infections, University of Liverpool, Liverpool, UK; 3. Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, UK; 4. Leeds Teaching Hospitals NHS Trust, Leeds, UK ; 5. Alderhey Children’s NHS Foundation Trust, Liverpool, UK; 6. The Walton Centre NHS Foundation Trust, Liverpool, UK;

## Corresponding author

Fiona McGill, fmcgill@liv.ac.uk

## Abstract 198 [200]

### Purpose of the review

The purpose of this review is to give an overview of viral meningitis and then focus in on some of the areas of uncertainty in diagnostics, treatment and outcome.

### Recent findings

Bacterial meningitis has been declining in incidence over recent years. Over a similar time period molecular diagnostics have increasingly been used. Due to both of these developments viral meningitis is becoming relatively more important. However, there are still many unanswered questions. Despite improvements in diagnostics many laboratories do not use molecular methods and even when they are used many cases still remain without a proven viral aetiology identified. There are also no established treatments for viral meningitis and the one potential treatment, aciclovir, which is effective in vitro for HSV, has never been subjected to a clinical trial.

### Summary

Viruses are in increasingly important cause of meningitis in the era of declining bacterial disease. The exact viral aetiology varies according to age and country. Molecular diagnostics can not only improve the rate of pathogen detection but also reduce unnecessary antibiotics use and length of hospitalisation. Further research is required into treatments for viral meningitis and the impact in terms of longer term sequelae.

## Keywords

Meningitis, Virus, Diagnostics, Therapeutics and Sequelae.

## Introduction

Viruses are responsible for many cases of meningitis every year, but are often overlooked as their sequelae are not as severe as bacterial meningitis or viral encephalitis.1,2 However, as bacterial meningitis decreases, secondary to vaccination, and the use of molecular diagnostics increases, viruses are being recognised as increasingly important.1-7 Meningitis occurs when there is inflammation of the meninges; viruses may reach the meninges from the bloodstream or be reactivated from a dormant state within the nervous system. If there is inflammation of the brain parenchyma itself this is encephalitis.

## Epidemiology

Many viruses can cause meningitis (table 1 and 2). The majority of cases are due to enteroviruses, herpesviruses or, in some parts of the world, arthropod-borne viruses (arboviruses). In unvaccinated populations mumps virus is also an important pathogen.8 The incidence of viral meningitis has been estimated between 0.26-17 cases per 100,000 dependent on the age or vaccination status of the population.1,9-13 The incidence is highest in young children where enteroviruses and parechoviruses account for most cases.5,14

There are up to 75,000 cases of enteroviral meningitis a year in the United States with the majority of infections occurring in the summer and autumn months.15 In the UK the annual incidence of enterovirus meningoencephalitis is estimated to be 2.13 per 100,000 for all age groups with a peak incidence of 312.5 per 100,000 in those under three months5. Enteroviruses can cause chronic meningoencephalitis in immunocompromised patients, especially those with antibody deficiencies.16 Without treatment it is often fatal.17 Enterovirus 71 is an important cause of neurological disease in some areas of the world. It usually causes hand, foot and mouth disease in children but a significant proportion of patients also develop neurological features including meningitis.18

The most frequently detected herpes viruses causing meningitis are herpes simplex virus type 2 (HSV-2) and varicella zoster virus (VZV).10,12,19 HSV-2 is usually sexually acquired, unlike herpes simplex virus type 1 (HSV-1) which is transmitted by oral to oral contact and causes sporadic encephalitis.20 Arboviruses are normally found in specific geographical areas depending on their vectors (figure 1). Most infections with arboviruses are subclinical21 but meningitis can occur, and is the most common neurological presentation of West Nile virus, Tick Borne Encephalitis (TBE) virus and Toscana virus.22-26 Other arboviruses such as Japanese encephalitis virus and dengue virus more commonly cause encephalitis but are also capable of causing meningitis27,28. The newly re-emerged Zika virus probably also causes meningitis, though its importance has not yet been assessed. HIV is an important cause of viral meningitis, normally during primary infection, as part of a seroconversion illness, but can occur in established infection.29,30 Up to 24% of primary HIV infection may present with meningitis.31-33 HIV may be present in 1 - 5% of culture negative meningitis.34,35 It is common in cases of meningitis to have no pathogen identified12,34,36 and HIV should always be included in the differential diagnosis.37

## Clinical Features

The clinical features of viral meningitis in adults are similar to those of bacterial meningitis and include headache, neck stiffness and photophobia (table 3).9,34,38 Only 10% of patients with HSV-2 meningitis will have genital lesions and approximately a third of patients will report a previous episode.39,40 Most of what was called Mollaret’s, or recurrent lymphocytic meningitis, is probably due to HSV-2, although other viruses have also been reported to cause repeated episodes of meningitis.41-48 VZV meningitis can occur in conjunction with primary infection (chickenpox), reactivation (shingles) or the use of the live attenuated vaccine49. It occurs without a rash in approximately 50% of cases.50-52 VZV meningitis is more common in older adults53.

Clinical features in children vary depending on the child’s age and the duration of illness; presenting features are often non-specific, particularly in infants and neonates, and include fever, poor feeding, irritability, lethargy, and vomiting. The presence of “classic” meningeal signs such as neck stiffness, bulging fontanelle or photophobia may increase the likelihood of meningitis in children.54 Seizures tend to occur less frequently in childhood viral meningitis, compared with bacterial.13,55 Presenting features also vary based on health-seeking behaviour. In Malawi, children with meningitis who had headaches, which is not regarded as a sign of severe illness, were often not brought to medical attention.56

## Diagnosis

Distinguishing viral meningitis from other causes of meningitis, in particular bacterial, on clinical grounds alone is difficult.57 A lumbar puncture (LP) is essential, not only to confirm meningitis as the cause for the patient’s symptoms, but also to identify the causative organism. Examination of the CSF usually shows a mildly elevated opening pressure; a raised total white cell count with lymphocyte predominance (neutrophils may predominate early on, especially in enteroviral disease); a moderately raised protein and a mildly reduced CSF:serum glucose ratio though normally still above 40%.

### Molecular Methods

The chances of obtaining a specific aetiological diagnosis in viral meningitis have increased since the advent of the polymerase chain reaction (PCR)5,10 and this is now the gold standard for diagnosis. Nucleic acid detection is more sensitive and quicker than traditional viral culture methods. 58,59 While culture can take up to 3 weeks, viral PCR can be run in a few hours. The sensitivity may be reduced if the PCR is performed very early in the course of disease, if the clinical manifestations occur after the virus has left the CSF or blood, or if the amount of virus is very low, as occurs in enteroviral meningitis. Stool samples may be positive for up to 3 weeks in enteroviral meningitis and might be more sensitive than CSF if symptoms have been present for more than 48 hours.60 Proving definitive causality can be difficult in these cases, as enterovirus is also excreted by healthy people.

PCR is available for all arboviruses but in some, such as TBE virus, the test may be negative by the onset of neurological symptoms. 61 TBE is a biphasic illness and although TBE viral RNA is detectable in blood during the initial phase, by the time the neurological features appear it has usually disappeared; antibody detection is more useful at this stage. Diagnosis of Toscana virus meningitis can be made by ELISA, immunofluorescence and/or neutralisation tests although PCR is increasingly used.62 West Nile viruses can be diagnosed by detecting IgM antibodies but correlation must be made with clinical and epidemiological features as there is cross reactivity with other flaviviruses as well as previous vaccination against Japanese Encephalitis of TBE.

### New Diagnostic Strategies

Despite improvements in diagnostic tests many patients (34-74%) with meningitis never have an aetiological diagnosis made12,36,63-65 Concern has been raised within Europe at the lack of standardised surveillance and diagnostic techniques potentially impairing our ability to identify emerging infections, particularly neuroinvasive arboviruses, in a timely fashion.66 Knowing the true burden of disease helps target future treatments, surveillance and prevention. Diagnosing a specific viral pathogen can reduce the length of antibiotics, the duration of hospital admission and the number of additional diagnostic tests requested.57,67,68 Even in current practice specific viral PCR is often not performed, despite proven diagnostic yield.69 A suggested practical diagnostic algorithm is given in figure 2.

Multiplex PCR is increasingly being employed in diagnostic laboratories. It uses conventional PCR technology but contains primers and probes for several pathogens so that many can be tested for at the same time. It standardises testing and reduces the need for the pre-selection by the clinician and improves pathogen detection when compared to individual PCR tests.70-72It has also been combined with microarray technology to increase the number of possible targets71,73,74 and may also reduce time and costs.70

Whilst conventional multiplex PCR addresses some of the problems associated with the diagnosis of viral meningitis and other neurological infections there is still a limit to the number of pathogens that can be detected. Newer techniques are being employed increasingly, both within and outside the research setting, which allow for the potential to improve our capabilities in pathogen detection and discovery.

A significant advance over the last decade has been the reduction in costs and the increase in speed of high throughput sequencing.75 This can be used to diagnose unexpected pathogens that were not being tested for by routine PCR such as Cache valley virus and Toscana virus.76-79 Whilst sequencing is currently largely used within research settings many are advocating its use within routine diagnostic microbiology,80 particularly in an outbreak scenario. Unbiased sequencing also has the potential to characterise the large number of cases of meningitis where no pathogen is currently identified. However it has not, as yet, been shown to offer any advantage over other molecular methods.81 Reasons for not identifying a pathogen in these cases may be due to low (or no) copies of nucleic acid in the CSF.

As well as increasing the number of targets on a diagnostic platform there is also a need to increase the speed of diagnosis. This could be achieved by moving toward point-of-care testing. Tests should be molecular or antigen based and with multiple pathogen targets, preferably pan-species detecting at least bacteria and viruses, with fungi and mycobacteria possibly also included. There should also be a move away from batching to single use, on demand tests. Microfluidic technology combines nucleic acid extraction and amplification to streamline the diagnostic process. A recent comparative study demonstrates the ability for HSV-1 or HSV-2 to be detected within 75 minutes of taking the CSF sample using a direct PCR technology with similar diagnostic accuracy to an in-house PCR.82 As well as direct testing from CSF there is also a need to be able to test for multiple pathogens or analytes at once. Newer cartridge based technologies incorporate extraction, amplification and detection in one system and are able to detect several different pathogens (bacteria, viruses and fungi) as well as increase pathogen detection when compared with routine practice.72Careful interpretation is required however, as with the increase in pathogen detection comes a possible increase in false positive results as well.

Loop-mediated isothermal amplification (LAMP) is an alternative method for DNA amplification. It is performed at a constant temperature and uses multiple primers to increase specificity. The amplified product is detected by changes in turbidity caused by an increasing amount of magnesium pyrophosphate in the reaction. It is quick, with the final product visible to the naked eye in less than two hours. This technique has good sensitivity for detection of *N. meningitidis, S. pneumoniae, H. influenzae* and *Mycobacterium tuberculosis*83-86and has been evaluated as a bedside test in the UK where it had a positive predictive value of 100% and a negative predictive value of 97% for detecting meningococcal disease.87 The speed and ease of diagnosis makes this a very attractive diagnostic tool and should be further evaluated in viral meningitis.88

All of the above mentioned diagnostic strategies focus on identifying the pathogen. However, these methods can only increase the diagnostic yield if there is a pathogen to detect. It is possible that the cause of meningitis in patients where no pathogen is found is para-infectious, post-infectious or even non-infectious. This is an area which requires further research. If the meningeal reaction is a para or post-infectious phenomenon examination of host responses, rather than pathogen detection, may be more useful in the future. These may take the form of serology, or perhaps gene expression. Additionally, researchers should consider the possibility that there may be non-infectious causes such as auto-immune. Further research on this is required to examine if there is a different cytokine or gene expression signature in those patients who have an identified virus (or bacteria) from those who have no identified aetiology. This would help to characterise this particular group of patients and start to identify if there might indeed by an alternative cause for the meningeal inflammation.

## Treatment

Despite diagnostic advances treatment options for viral meningitis are lacking. Pleconaril showed some promise as a treatment for enteroviral neurological infection, in the 1990s and 2000s, as it achieved high CSF concentrations. However studies in acute meningitis showed only a slight reduction in the duration of headache and due to the potential for drug interactions it was never licensed.89 It had some degree of success in immunocompromised patients with chronic enteroviral infection, 90 but has no activity against EV71. Recently interest has peaked again showing potential for its use in neonatal enteroviral sepsis91. As most patients with enteroviral meningitis recover quickly with minimal sequelae the need for a specific antiviral treatment is debated. It is known that patients with enteroviral meningitis can suffer from prolonged headaches and it may be this particular symptom that any treatment would aim to reduce. Further assessment of the longer term sequelae, in particular any effects on quality of life and productivity, should be undertaken in order to ascertain if a treatment for enteroviral meningitis would be warranted.

Although aciclovir has proven activity against herpes viruses in-vitro, and is used in HSV encephalitis and genital HSV disease, its role in acute HSV meningitis remains unclear. To date, there have been no randomised controlled trials or other studies to help guide clinicians. Current management is highly variable and ranges from no specific antiviral treatment to three weeks of intravenous aciclovir.39,92 A properly conducted trial is needed. Potential benefits of aciclovir treatment that may be assessed by such a trial include shorter duration of symptoms, reduction in any potential sequelae and less recurrences. Conversely if a trial were to show no beneficial effect of aciclovir on the individual this could also be valuable. Aciclovir can be nephrotoxic and often results in patients being admitted to hospital for longer than those who are not treated. Therefore, if aciclovir was shown not to be efficacious potential benefits would include reducing the number of patients on potentially toxic drugs, reducing healthcare associated costs via a reduction in the length of hospitalisation.

Another often asked question is whether to treat recurrent HSV-2 meningitis prophylactically with aciclovir. Some experts recommend prophylaxis whilst others refrain. Although aciclovir prophylaxis has proven useful in preventing frequent recurrences of genital herpes,93 a double blind randomised placebo controlled trial showed no benefit of giving oral valaciclovir (the prodrug of aciclovir), at a dose of 500mg twice daily, in preventing recurrences of HSV-2 meningitis.94 Patients were treated for a year and then followed up one year later. Patients in the valaciclovir group had a significantly higher rate of recurrences than the placebo group in the year following treatment. Even during the treatment year these patients had a non-significantly higher rate of recurrences. The authors postulate that the dose may not have been appropriate and that the higher rate of recurrences off the drug may be due to a rebound phenomenon. As with HSV there is no evidence base on which to recommend the use of aciclovir in VZV meningitis and decisions need to be made on a case by case basis. Aciclovir is recommended where there is evidence of vasculopathy or encephalitis, and also used if meningitis co-exists with radiculopathy or severe shingles.95,96

## Conclusions

Viruses are a major cause of meningitis in the era of conjugate bacterial vaccination. Despite the prevalence of the condition there are still many gaps in knowledge, especially with regard to diagnosis and treatment.

Many patients with a clinical illness compatible with viral meningitis and a CSF pleocytosis never have an aetiological agent identified. Given the changing epidemiology of viral meningitis and the emergence and spread of new viruses such as West Nile, Zika and Toscana viruses clinicians need to be vigilant. Current diagnostics may be inadequate and research should focus on developing new, rapid tests, potenially using technology other than PCR.

There are no treatments for almost all causes of viral meningitis. Although mortality may not be a significant issue, antivirals may reduce morbidity and clinical trials should assess this. In the case of herpes meningitis, demonstration of no benefit of aciclovir may be just as useful as any benefits.

## Key points

* Viral meningitis it the most common form of meningitis in many countries
* Many cases of viral meningitis do not have a proven pathogen identified
* Diagnosing a specific pathogen can reduce the use of unnecessary antibiotic, and improve outcomes by decreasing length of hospitalisation and reducing costs.
* There are no proven treatments for viral meningitis and a trial assessing the efficacy of aciclovir in herpes meningitis is needed
* Research is needed to document the longer term outcomes of viral meningitis including the health economic impact.

### Acknowledgement

We would like to thank Mr Richard Crew with his assistance in drawing the arboviral maps.

### Financial Support

FM is a National Institute for Health Research (NIHR) doctoral research fellow and TS is an NIHR senior investigator. Both receive support from the NIHR. All authors are members of the NIHR Health Protection Research unit in Emerging and Zoonotic Infections. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, the Department of Health or Public Health England. This work was conducted independently of influence from the NIHR.

### Conflicts of interest

None

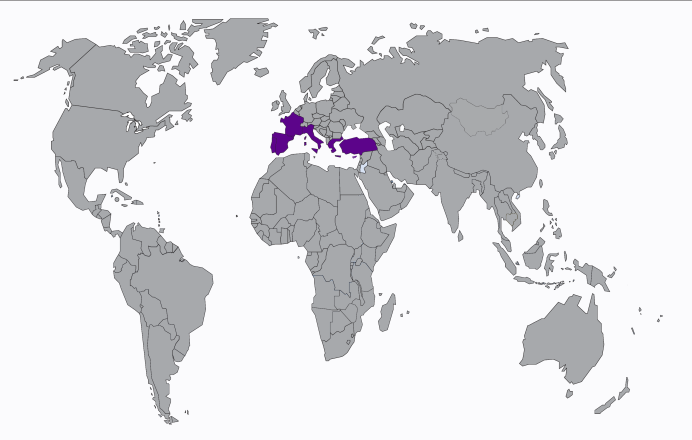
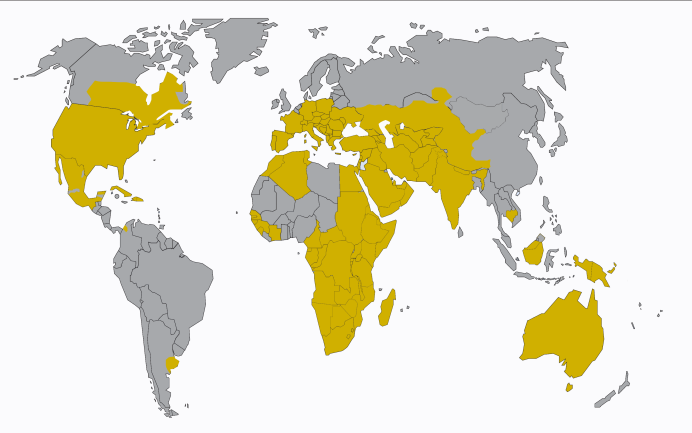
|  |  |  |  |
| --- | --- | --- | --- |
| Table 1. Viral causes of meningitis | | | |
|  | Species | | |
| Common | Less common | Rare |
| Picornaviruses | Echoviruses  Coxsackie viruses  Enterovirus 71 |  | Parechoviruses\*\*\* |
| Herpesviridae | Herpes simplex virus type 2  Varicella zoster virus |  | Epstein Barr Virus\*\*  Cytomegalovirus\*\*  Herpes simplex virus type 1  Human herpes virus 6\*\*  Human herpes virus 7 |
| Arboviruses | Toscana virus\*  West Nile virus\* | Tick borne encephalitis virus\* | Japanese encephalitis virus  Dengue virus |
| Others |  | Human immunodeficiency virus  Mumps virus | Lymphocytic choriomeningitis virus |
| \*Geographically dependent \*\*Consider in immunocompromised \*\*\*Consider in very young children | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Table 2. Features of viral causes of meningitis | | | | | | |
| Virus | Viral genus | Structure and genome | Mode of transmission | Vector | Diagnosis | Other comments |
| HSV-2 | Herpesvirus | Enveloped, double stranded DNA virus | Sexually | n/a | CSF PCR |  |
| VZV | Herpesvirus | Enveloped, double stranded DNA virus | Airborne | n/a | CSF PCR |  |
| Enteroviruses | Picornavirus | Non enveloped, single stranded RNA virus | Faecal-oral | n/a | CSF PCR | (stool sample for PCR may be helpful if symptoms for >48 hours) |
| TBEV | Flavivirus | Enveloped, positive sense single stranded RNA virus | Arthropod borne | *Ixodes* Tick | TBEV serum IgM | Intrathecal antibodies rise approximately 10 days after serum. |
| Toscana virus | Bunyavirus | Enveloped, negative sense single strand RNA virus | Arthropod borne | Phlebotomine sandfly | CSF PCR |  |
| WNV | Flavivirus | Enveloped, positive sense single stranded RNA virus | Arthropod borne | Mosquito (several species) | Serum or CSF IgM | Cross reactivity with other flaviviruses and TBE or JE vaccination. |
| HSV-2=Herpes-simplex virus type 2. VZV = Varicella zoster virus. TBEV = Tick Borne Encephalitis Virus. WNV = West Nile Virus, CSF = Cerebrospinal Fluid. PCR = Polymerase Chain Reaction. JE = Japanese Encephalitis | | | | | | |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 3. Clinical and laboratory features associated with different aetiologies of viral meningitis in adults | | | | | | | | | | | | | | |
|  | **Enterovirus** | | | **Herpes Simplex Virus Type 2** | | | | | | **Varicella Zoster Virus** | | | | **All viruses** |
|  | Ihekwaba et al | Nowak et al | **Summary measure (mean)** | Ihekwaba et al | Nowak et al | | Omland et al | O’Sullivan et al | **Summary measure (mean)** | Ihekwaba et al | Nowak et al | **Summary measure (mean)** | | **Summary measure (mean)** |
| Headache n/N (%) | 22/22 (100) | 19/19 (100) | **100%** | 8/8 (100) | 2/2 (100) | | 43/43 (100) | 72/76 (95) | **98.75%** | 6/8 (75) | 2/2 (100) | **87.5%** | | **96.7%** |
| Photophobia n/N (%) | 18/22 (82) | NR | **82%** | 5/8 (62.5) | NR | | 22/43 (51) | 42/76 (55) | **56.2%** | 2/8 (25) | NR | **25%** | | **56.7%** |
| Neck Stiffness n/N (%) | 17/22 (77) | 9/19 (47) | **62%** | 8/8 (100) | 1/2 (50) | | 35/43 (81) | 52/76 (68) | **74.75%** | 3/8 (37.5) | 1/2 (50) | **43.75%** | | **70%** |
| Nausea/Vomiting n/N (%) | 20/22 (91) | 14/19 (74) | **82.5%** | 8/8 (100) | 0 | | 29/43 (67) | 36/76 (47) | **71.3%** | 4/8 (50) | 0/2 (0%) | **50%** | | **62.4%** |
|  | | | **Total Range** |  | | | | | **Total Range** |  | | **Total Range** | | **Total Range** |
| CSF WCC x 10^6/L, median (range) | 51 (0-1298) | 236(13-670) | **0-1298** | 240(180-2200) | 293(186,400) | | 146.2 (2-270) | 238 (2-1900) | **180-2200** | 207(6-450) | 43(15,71) | **6-450** | | **0-2200** |
| Percentage lymphocytes, median (range) | 91 (5-100) | NR\* | **5-100** | 100 (80-100) | NR\* | | NR | NR | **80-100** | 100 (90-100) | NR\* | **90-100** | | **5-100** |
| CSF Protein mg/L, median (range) | 640 (100-875) | 800(270-3380) | **100-3380** | 1205 (611-3704) | 525 (520,530) | | 131(36-273) | 116(23-996) | **23-3704** | 974 (581-2616) | 450 (340,550) | **340-2616** | | **23-3704** |
| CSF:serum glucose ratio, median (range) | 0.6 (0.26-0.76) | NR | **0.26-0.76** | 0.48(0.47-0.67) | NR | | NR | NR | **0.47-0.67** | 0.55(0.4-0.73) | NR | **0.4-0.73** | | **0.26-0.76** |
| CSF glucose, mMol/L (range) | NR | NR | NR | NR | | NR | 3.07 (1.4-4.2) | 3.05 (1.7-7.4) | **1.4-7.4** | NR | NR | | NR | **1.4-7.4** |
| EV=Enterovirus HSV=Herpes Simplex Virus VZV = Varicella Zoster Virus NR = Not Reported \*Documented as lymphocytic CSF monocytosis present in all | | | | | | | | | | | | | | |

**Figure 1. Geographic distribution of important arboviruses that cause meningitis.**

1. **West Nile virus, b) Toscana virus, c) Tick borne encephalitis virus**



c

b

a

**Figure 2. Diagnostic algorithm for suspected viral meningitis.**

Clinical History

Acute onset of meningeal symptoms i.e. headache, neck stiffness and/or photophobia without altered consciousness or change in behaviour

Sample type

Cerebrospinal Fluid

Blood

Initial Testing

* Cell count
* Culture
* Protein
* Glucose
* Full blood count
* C-reactive protein
* Renal function
* Clotting screen
* Paired serum glucose

Pathogen identification – 1st line

PCR for HSV-1, HSV- 2, VZV, enterovirus and Parechovirus\*

HIV test

Other

* Stool sample for Enterovirus PCR
* Throat sample for Enterovirus PCR

Pathogen identification – 2nd line

PCR for, EBV, CMV, Mumps, HHV-6 and 7.

Pathogen identification – 3rd line or if travel history suggestive.

PCR for Toscana virus, Tick borne encephalitis virus and West nile virus.

* Mumps IgM
* Enterovirus IgM

\*Parechovirus should be performed in infants under 3 months of age

## References

Papers of particular interest have been highlighted as:

\* of special interest

\*\* of outstanding interest

1. Khetsuriani N, Quiroz ES, Holman R, Anderson LJ. Viral meningitis-associated hospitalisations in the united states, 1988-1999. *Neuroepidemiology* 2003; **22**: 345-52.

\*\* *The largest epidemiological study examining viral meningitis*

2. Magazzini S, Nazerian P, Vanni S, et al. Clinical picture of meningitis in the adult patient and its relationship with age. *Intern Emerg Med* 2012; **7**: 359-64.

3. Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the united states, 1998-2007. *N Engl J Med* 2011; **364**: 2016-25.

4. Okike IO, Ribeiro S, Ramsay M, Heath PT, Sharland M, Ladhani SN. Trends in bacterial, mycobacterial and fungal meningitis in england and wales 2004-11: An observational study. *Lancet Infect Dis* 2014; **14**: 301-7.

5. Kadambari S, Okike I, Ribeiro S, et al. Seven-fold increase in viral meningo-encephalitis reports in england and wales during 2004-2013. *Journal of Infection* 2014; **69**: 326-32.

*\*\* Laboratory based study looking at the rise in reports of viral meningitis with the implementation of PCR testing.*

6. McBride S, Fulke J, Giles H, et al. Epidemiology and diagnostic testing for meningitis in adults as the meningococcal epidemic declined at middlemore hospital. *New Zealand Medical Journal* 2015; **128**: 17-24.

7. Martin NG, Iro MA, Sadarangani M, Goldacre R, Pollard AJ, Goldacre M. Hospital admissions for viral meningitis in children in england over five decades: A population-based observational study. *Lancet Infect Dis* 2016: Published online August 12 2016.

*\* A study interrogating various databases to examine the role viral meningitis has played over the last half a century.*

8. Xie Y, Tan Y, Chongsuvivatwong V, et al. A population-based acute meningitis and encephalitis syndromes surveillance in guangxi, china, may 2007-june 2012. *PLoS One* 2015; **10**(12): e0144366.

9. Omland LH, Vestergaard BF, Wandall JH. Herpes simplex virus type 2 infections of the central nervous system: A retrospective study of 49 patients. *Scand J Infect Dis* 2008; **40**: 59-62.

10. Koskiniemi M, Rantalaiho T, Piiparinen H, et al. Infections of the central nervous system of suspected viral origin: A collaborative study from finland. *Journal of Neurovirology* 2001; **7**: 400-8.

11. Rantakallio P, Leskinen M, Von Wendt L. Incidence and prognosis of central nervous system infections in a birth cohort of 12000 children. *Scand J Infect Dis* 1986; **18** 287-94.

12. Kupila L, Vuorinen T, Vainionpaa R, Hukkanen V, Marttila RJ, Kotilainen P. Etiology or aseptic meningitis and encephalitis in an adult population. *Neurology* 2006; **66**: 75-80.

13. Michos AG, Syriopoulou VP, Hadjichristodoulou C, et al. Aseptic meningitis in children: Analysis of 506 cases. *PLoS One* 2007; **2**(7): e674.

14. Messacar K, Schreiner TL, Maloney J, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus d68 in children in colorado, USA. *Lancet* 2015; **385**: 1662-71.

15. Rotbart HA. Enteroviral infections of the central nervous system. *Clin Infect Dis* 1995; **20**: 971-81.

16. McKinney RE, Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinaemic patients. *Rev Infect Dis* 1987; **9**(2): 334-56.

17. Misbah SA, Spickett GP, Ryba PCJ, et al. Chronic enteroviral meningoencephalitis in agammaglobulinaemia:Case report and literature review. *J Clin Immunol* 1992; **12**(4): 266-70.

18. Ooi MH, Wong SC, Podin Y, et al. Human enterovirus 71 disease in sarawak, malaysia: A prospective clinical, virological, and molecular epidemiological study. *Clin Infect Dis* 2007; **44**: 646-56.

19. Hausfater P, Fillet AM, Rozenberg F, et al. Prevalence of viral infection markers by polymerase chain reaction amplification and interferon-alpha measurements among patients undergoing lumbar puncture in an emergency department. *J Med Virol* 2004; **73**: 137-46.

20. Granerod J, Ambrose H, Davies N, et al. Causes of encephalitis and differences in their clinical presentations in england: A multicentre, population-based prospective study. *Lancet* 2010; **10**: 835-43.

21. Gustafson R, Svenungsson B, Forsgren M, Gardulf A, Granstrom M. Two-year survey of the incidence of lyme borreliosis and tick borne encephalitis in a high risk population in sweden. *Eur J Clin Microbiol* 1992; **11**(10): 894-900.

22. Lesnicar G, Poljak M, Seme K, Lesnicar J. Pediatric tick-borne encephalitis in 371 cases from an endemic region in slovenia, 1959 to 2000. *Pediatr Infect Dis J* 2003; **22**: 612-7.

23. Gunther G, Haglund M, Lindquist L, Forsgren M, Skoldenberg B. Tick-borne encephalitis in sweden in relation to aseptic meningo-encephalitis of other etiology:A prospective study of clinical course and outcome. *J Neurol* 1997; **244**: 230-8.

24. Kaiser R. The clinical and epidemiological profile of tick-borne encephalitis in southern germany 1994-1998 - a prospective study of 656 patients. *Brain* 1999; **122**: 2067-78.

25. Bode AV, Sejvar JJ, Pape WJ, Campbell GL, Marfin AA. West nile virus disease: A descriptive study of 228 patients hospitalized in a 4-county region of colorado in 2003. *Clin Infect Dis* 2006; **42**: 1234-40.

26. Dupouey J, Bichaud L, Ninove L, et al. Toscana virus infections: A case series from france. *Journal of Infection* 2014; **68**(3): 290-5.

27. Ooi MH, Lewthwaite P, Lai BF, et al. The epidemiology, clinical features, and long-term prognosis of japanese encephalitis in central sarawak, malaysia, 1997–2005. *Clinical Infectious Diseases* 2008; **47**(4): 458-68.

28. Araújo F, Nogueira R, Araújo MdS, et al. Dengue in patients with central nervous system manifestations, brazil. *Emerging Infectious Diseases* 2012; **18**(4): 677-9.

29. Hollander H, Stringari S. Human immunodeficiency virus-associated meningitis. *The American Journal of Medicine* 1987; **83**(5): 813-6.

30. Bekondi C, Bernede C, Passone N, et al. Primary and opporunistic pathogens associated with meningitis in adults in bangui, central african republic, in relation to human immunodeficiency virus serostatus. *International Journal of Infectious Diseases* 2006; **10**: 387-95.

31. Ho DD, Sarngadharan MG, Resnick L, Dimarzo-Veronese F, Rota TR, Hirsch MS. Primary human t-lymphotropic virus type iii infection. *Ann Intern Med* 1985; **103**(6): 880-3.

32. Boufassa F, Bachmeyer C, Carre N, et al. Influence of neurologic manifestations of primary human immunodeficiency virus infection on disease progression. *J Infect Dis* 1995; **171**: 1190-5.

33. Schaker T, Collier AC, Hughes J, Shea T, Corey L. Clinical and epidemiological features of primary hiv infection. *Ann Intern Med* 1996; **125**(4): 257-64.

34. Nowak DA, Boehmer R, Fuchs HH. A retrospective clinical, laboratory and outcome analysis in 43 cases of acute aseptic meningitis. *Eur J Neurol* 2003; **10**: 271-80.

35. Hanson KE, Reckleff J, Hicks L, Castellano C, Hicks CB. Unsuspected hiv infection in patients presenting with acute meningitis. *Clin Infect Dis* 2008; **47**: 433-4.

36. de Ory F, Avellon A, Echevarria JE, et al. Viral infections of the central nervous system in spain: A prospective study. *J Med Virol* 2013; **85**: 554-62.

*\*\* A large comprehensive study evaluating the aetiology of neurological infections in both children and adults.*

37. British HIV Association, British Association of Sexual Health and HIV, British Infection Society. Uk national hiv testing guidelines for hiv testing 2008. 2008.

38. Ihekwaba UK, Kudesia G, McKendrick M. Clinical features of viral meningitis in adults: Significant differences in cerebrospinal fluid findings among herpes simplex virus, varicella zoster virus and enterovirus infections. *Clin Infect Dis* 2008; **47**: 783-9.

39. Landry ML, Greenwold J, Vikram HR. Herpes simplex type-2 meningitis: Presentation and lack of standardized therapy. *Am J Med* 2009; **122**: 688-91.

40. O'Sullivan CE, Aksamit A, Harrington J, Harmsen WS, Mitchell S, R. P. Clinical spectrum and laboratory characteristics associated with detection of herpes simplex virus DNA in cerebrospinal fluid. *Mayo Clin Proc* 2003; **78**: 1347-52.

41. Mollaret P. La meningite endothelio-leukocytaire multi-recurrente benigne. *Rev Neurol (Paris)* 1944; **76**: 57-67.

42. Capouya JD, Berman DM, Dumois JA. Mollaret's meningitis due to human herpesvirus 6 in an adolescent. *Clin Pediatr* 2006; **45**: 861-3.

43. Yamamoto LJ, Tedder DG, Ashley R, Levin MJ. Herpes simplex virus type 1 DNA in cerebrospinal fluid of a patient with mollaret's meningitis. *N Engl J Med* 1991; **325**: 1082-5.

44. Takeuchi M, Yamane K, Kobayashi I, Maruyama S. A case of recurrent epstein-barr virus meningitis (japanese). *Rinsho Shinkeigaku - Clinical Neurology* 1989; **29**(1): 85-8.

45. Graman PS. Mollaret's meningitis associated with acute epstein-barr virus mononucleosis. *Arch Neurol* 1987; **44**(11): 1204-5.

46. Tedder DG, Ashley R, Tyler KL, Levin MJ. Herpes simplex virus infection as a cause of benign recurrent lymphocytic meningitis. *Ann Intern Med* 1994; **121**: 334-8.

47. Kupila L, Vainionpaa R, Vuorinen T, Marttila RJ, Kotilainen P. Recurrent lymphocytic meningitis. *Arch Neurol* 2004; **61**: 1553-7.

48. Kallio-Laine K, Seppanen M, Kautiainen H, et al. Recurrent lymphocytic meningitis positive for herpes simplex virus type 2. *Emerg Infect Dis* 2009; **15**: 1119-22.

49. Levin MJ, De Biasi RL, Bostik V, Schmid DS. Herpes zoster with skin lesions and meningitis caused by 2 different genotypes of the oka varicella-zoster virus vaccine. *J Infect Dis* 2008; **198**: 1444-7.

50. Persson A, Bergstrom T, Lindh M, Namvar L, Studahl M. Varicella-zoster virus cns disease - viral load, clinical manifestations and sequels. *J Clin Virol* 2009; **46**: 249-53.

51. Pahud BA, Glaser CA, Dekker CL, Arvin AM, Schmid DS. Varicella zoster disease of the central nervous system: Epidemiological, clinical and laboratory features 10 years after the introduction of the varicella vaccine. *J Infect Dis* 2011; **203**: 316-23.

52. Chamizo FJ, Gilarranz R, Hernandez M, Ramos D, Pena MJ. Central nervous system infections caused by varicella-zoster virus. *J Neurovirol* 2016.

53. Parisi SG, Basso M, Del Vecchio C, et al. Viral infections of the central nervous system in elderly patients: A retrospective study. *Int J Infect Dis* 2016; **44**: 8-10.

54. Curtis S, Stobart K, Vandermeer B, Simel DL, Klassen T. Clinical features suggestive of meningitis in children: A systematic review of prospective data. *Pediatrics* 2010; **126**: 652-960.

55. Molyneux E, Riordan FAI, Walsh A. Acute bacterial meningitis in children presenting to the royal liverpool children's hospital, liverpool, uk and the queen elizabeth central hospital in blantyre, malawi: A world of difference. *Annals of Tropical Paediatrics* 2006; **26**(1): 29-37.

56. Desmond NA, Nyirenda D, Dube Q, et al. Recognising and treatment seeking for acute bacterial meningitis in adults and children in resource-poor settings: A qualitative study. *PLoS ONE* 2013; **8**(7): e68163.

57. Chadwick D, Lever A. The impact of new diagnostic methodologies in the management of meningitis in adults at a teaching hospital. *QJM* 2002; **95**: 663-70.

58. Aurelius E, Johansson B, Skoldenberg B, Staland A, Forsgren M. Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal spinal fluid. *Lancet* 1991; **337**(8735): 189-92.

59. Carrol E, Beadsworth M, Jenkins N, et al. Clinical and diagnositc findings of an echovirus meningitis outbreak in the north west of england. *Postgrad Med J* 2006; **82**: 60-4.

60. Kupila L, Vuorinen T, Vainionpaa R, Marttila R J, Kotilainen P. Diagnosis of enteroviral meningitis by use of polymerase chain reaction of cerebrospinal fluid, stool and serum specimens. *Clin Infect Dis* 2005; **40**: 982-7.

61. Stockl-Puchhammer E, Kunz C, Mandl CW, Heinz FX. Identification of tick-borne encephalitis virus ribonucleic acid in tick suspensions and in clinical specimens by a reverse transcription-nested polymerase chain reaction assay. *Clin Diagn Virol* 1995; **4**(4): 321-6.

62. Brisbarre N, egrave, ge, et al. A rapid and specific real time rt-pcr assay for diagnosis of toscana virus infection. *Journal of clinical virology* 2015; **66**(1): 107-11.

63. Harrell T, Hammes JS. Meningitis admitted to a military hospital: A retrospective case series. *Military Medicine* 2012; **177**(10): 1223-6.

64. Michael B, Sidhu M, Stoeter D, et al. Acute central nervous system infections in adults—a retrospective cohort study in the nhs north west region. *QJM* 2010; **103**: 749-58.

65. Han SH, Choi HY, Kim JM, Park KR, Youn YC, Shin HW. Etiology of aseptic meningitis and clinical characteristics in immune-competent adults. *J Med Virol* 2016; **88**(1): 175-9.

66. Donoso Mantke O, Vaheri A, Ambrose H, et al. Analysis of the surveillance situation for viral encephalitis and meningitis in europe. *Eurosurveillance* 2008; **13**(3): 1-10.

67. Ramers C, Billman G, Hartin M, Ho S, Sawyer M. Impact of a diagnostic cerebrospinal fluid enterovirus polymerase chain reaction test on patient management. *JAMA* 2000; **283**(20): 2680-5.

68. Robinson CC, Willis M, Meagher A, Gieseker KE, Rotbart H, Glode MP. Impact of rapid polymerase chain reaction results on management of pediatric patients with enteroviral meningitis. *Pediatr Infect Dis J* 2002; **21**: 283-6.

*\*\* Study showing the benefit of diagnosing a pathogen on reducing costs and other investigations.*

69. Nesher l, Hadi CM, Salazar l, et al. Epidemiology of meningitis with a negative csf gram stain: Under-utilization of available diagnostic tests. *Epidemiol Infect* 2015; **FirstView**: 1-9.

70. Bennett S, Carman WF, Gunson RN. The development of a multiplex real-time pcr for the detection of herpes simplex virus 1 and 2, varicella zoster virus, adenovirus and *chlamydia trachomatis* from eye swabs. *J Virol Methods* 2012; **189**: 143-7.

71. Boving MK, Pedersen LN, Moller JK. Eight-plex pcr and liquid-array detectino of bacterial and viral pathogens in cerebrospinal fluid from patient with suspected meningitis. *J Clin Micro* 2009; **47**(4): 908-13.

72. Wootton SH, Aguilera E, Salazar L, Hemmert AC, Hasbun R. Enhancing pathogen identification in patients with meningitis and a negative gram stain using the biofire filmarray meningitis/encephalitis panel. *Ann Clin Microbiol Antimicrob* 2016; **15**: 26.

73. Mannonen L, Vainionpaa R, Kauppinen J, et al. Evaluation of multpilex polymerase chain reaction and microarray-based assay for rapid herpesvirus diagnostics. *Diagn Micr Infect Dis* 2012; **73**: 74-9.

74. Leveque N, Van Haecke, Renois F, Boutolleau D, Talmud D, Andreoletti L. Rapid virological diagnosis of central nervous system infections by use of a multiplex reverse transcription-pcr DNA microarray. *J Clin Micro* 2011; **49**(11): 3874-9.

75. Lipkin WI. The changing face of pathogen discovery and surveillance. *Nature Reviews Microbiology* 2013.

76. Avellon A, Rubio G, Palacios G, et al. Enterovirus 75 and aseptic meningitis, spain 2005. *Emerg Infect Dis* 2006; **12**(10): 1609-11.

77. Perez-Ruin M, Navarro-Mari JM, Sanchez-Seco MP, et al. Lymphocytic choriomeningitis virus-associated meningitis, southern spain. *Emerg Infect Dis* 2012; **18**(5): 855-8.

78. Nguyen NL, Zhao G, Hull R, et al. Cache valley virus in a patient diagnosed with aseptic meningitis. *J Clin Micro* 2013; **51**: 1966-9.

79. Cordey S, Bel M, Petty TJ, et al. Toscana virus meningitis case in switzerland: An example of the ezvir bioinformatics pipeline utility for the identification of emerging viruses. *Clinical Microbiology and Infection (Wiley)* 2015; **21**(4): 387.e1-.e4.

80. Torok ME, Peacock SJ. Rapid whole-genome sequencing of bacterial pathogens in the clinical microbiology laboratory-pipe dream or reality? *J Antimicrob Chemother* 2012; **67**: 2307-8.

81. Hsu CC, Tokarz R, Briese T, Tsai HC, Quan PL, Lipkin WI. Use of staged molecular analysis to determine causes of unexplained central nervous system infections. *Emerg Infect Dis* 2013.

82. Kuypers J, Boughton G, Chung J, et al. Comparison of the simplexa hsv1 and 2 direct kit and laboratory developed real time pcr assays for herpes simplex virus detection. *J Clin Virology* 2015; **62**: 103-5.

83. Nagdev KJ, Kashyap RS, Parida MM, et al. Loop-mediated isothermal amplification for rapid and reliable diagnosis of tuberculous meningitis. *J Clin Microbiol* 2011; **49**(5): 1861-5.

84. Kim DW, Kilgore PE, Kim EJ, et al. The enhanced pneumococcal lamp assay: A clinical tool for the diagnosis of meningitis due to *streptococcus pneumoniae*. *PLoS ONE* 2012; **7**(8): e42954.

85. Lee D, Kim EJ, Kilgore PE, et al. Clinical evaluation of a loop-mediated isothermal amplification (lamp) assay for rapid detection of neisseria meningitidis in cerebrospinal fluid. *PLoS One* 2015; **10**(4): e0122922.

86. Kim DW, Kilgore PE, Kim EJ, Kim SA, Anh DD, Seki M. Loop-mediated isothermal amplification assay for detection of haemophilus influenzae type b in cerebrospinal fluid. *J Clin Microbiol* 2011; **49**(10): 3621-6.

87. Bourke TW, McKenna JP, Coyle PV, Shields MD, Fairley DJ. Diagnostic accuracy of loop-mediated isothermal amplification as a near-patient test for meningococcal disease in children: An observational cohort study. *Lancet Infect Dis* 2015; **15**(5): 552-8.

88. Jaianand K, Saravanan N, Gunasekaran P, Sheriff AK. Development of a new method for diagnosis of group b coxsackie genome by reverse transcription loop-mediated isothermal amplification. *Indian J Med Microbiol* 2011; **29**(2): 110-7.

89. Desmond RA, Accortt NA, Talley L, Villano A, Soong SJ, Whitley RJ. Enteroviral meningitis: Natural history and outcome of pleconaril therapy. *Antimicrob Agents Ch* 2006; **50**(7): 2409-14.

90. Rotbart H, Webster DA. Treatment of potentially life-threatening enterovirus infections with pleconaril. *Clin Infect Dis* 2001; **32**: 228-35.

91. Abzug MJ, Michaels MG, Wald E, et al. A randomized, double-blind, placebo-controlled trial of pleconaril for the treatment of neonates with enterovirus sepsis. *J Pediatric Infect Dis Soc* 2016; **5**(1): 53-62.

92. Kaewpoowat Q, Salazar L, Aguilera E, Wootton SH, Hasbun R. Herpes simplex and varicella zoster cns infections: Clinical presentations, treatments and outcomes. *Infection* 2016; **44**(3): 337-45.

*\* Recent clinical study evaluating the herpes viruses and their role in neurological infections.*

93. Patel R, Bodsworth NJ, Wooley P, et al. Valaciclovir for the suppression of recurrent genital hsv infection: A placebo controlled study of once daily therapy. *Genitourin Med* 1997; **73**: 105-9.

94. Aurelius E, Franzen-Rohl E, Glimaker M, et al. Long term valacyclovir suppressive treatment after herpes simplex virus type-2 meningitis:A double blind, randomized controlled trial. *Clin Infect Dis* 2012; **54**: 1304-13.

95. Steiner I, Spivack J, Deshmane S, Ace C, Preston C, Fraser N. A herpes simplex virus type 1 mutant containing a nontransinducing vmw65 protein establishes latent infection in vivo in the absence of viral replication and reactivates efficiently from explanted trigeminal ganglia. *J Virol* 1990; **64**(4): 1630-8.

96. Romero JR, Newland JG. Viral meningitis and encephalitis: Traditional and emerging viral agents. *Semin Paediatr Infect Dis* 2003; **14**(2): 72-82.

97. Ooi MH, Wong SC, Mohan A, et al. Identification and validation of clinical predictors for the risk of neurological involvement in children with hand, foot and mouth disease in sarawak. *BMC Infect Dis* 2009; **9**(3).

98. Wang S, Lei H, Huang M, et al. Modulation of cytokine production of intravenous immunoglobulin in patients with enterovirus 71-associated brainstem encephalitis. *J Clin Virology* 2006; **37**: 47-52.

99. Mickiene A, Laiskonis A, Gunther G, Vene S, Lundkvist A, Lindquist L. Tickborne encephalitis in an area of high endemnicity in lithuania: Disease severity and long term prognosis. *Clin Infect Dis* 2002; **35**: 650-8.